The specimens here described are in many of their general features similar to *B. perichitinea* and are, therefore, regarded as representatives of the same genus. They differ in many respects, however, from the genotype as described by Gebauer and, therefore, are regarded as representing a new species.

It is conceded that certain of the described differences between *B. wilsoni* and *B. perichitinea* are of possible generic value. Notable among them are discrepancies in the number of cephalic papillae and in the nature of the buccal cavity and of the anterior end of the esophagus, and, corollary to the last, in the derivation, position, and orientation of the dorsal onchium and the denticles associated with it; also in this category are the presence in *B. wilsoni* of a circumoral elevation and a rudimentary leaf crown. However, the writer suspects that reexamination of the type specimens of *B. perichitinea* may reveal a closer similarity and relationship to *B. wilsoni* in these respects than now is evident.

In addition to the differences thus far alluded to, *B. wilsoni* is distinguished from *B. perichitinea* by presence of preburSal papillae and a telemon, absence of cervical alae, less marked inequality in the depth of the clefts between the dorsal and lateral lobes of the bursa, lack of dextral curvature and an accessory ventral rodlike process in the dorsal ray, longer spicules of different shape and orientation, larger gubernaculum, larger females with more anteriorly situated vulva, greater number of longitudinal cuticular ridges, shorter dorsal onchium, mediolateral and posteriorlateral rays longer than externolaterals, and lateroventral rays thicker than externodorsals. There also appear to be differences in the extent of the internal chitinized processes in the caudal region, notably, the absence in *B. wilsoni* of a narrow process extending between the spicules and the anterior extremity of the dorsal process, as well as absence of the pair of broom-shaped lateral processes, figured for *B. perichitinea*.

ZOOLOGY.—*Observations on the route of migration of the common liver fluke,* Fasciola hepatica, *in the definitive host.*[1]

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The essentials of the life history of the common liver fluke, *Fasciola hepatica*, have been known since 1882, when Thomas and Leuckart, independently, showed that the snail *Lymnaea truncatula* served as an intermediate host of this important parasite. In spite of these and subsequent investigations there still remain details concerning the development of the fluke in the intermediate and definitive hosts that have not been fully worked out. Important among these is the route of migration to the liver of the young fluke after its excystment in the digestive tract of the definitive host.

Three possible routes of migration have been postulated, namely, (1) direct migration from the intestine to the bile ducts through the hepatic duct; (2) passive transportation by the portal circulation after penetration of the intestinal mucosa, the young fluke gaining access to the bile ducts by perforation; and (3) penetration of the intestine, active migration in the peritoneal cavity, perforation of the liver capsule, and migration through the liver parenchyma to the bile ducts. The first of these possible routes is the one most generally accepted, although it is the only one entirely unsupported by experimental evidence. On the other hand, Bugge (1935) concluded, on the basis of his examination of numerous infected calves, that the young flukes reached the liver via the portal system. Sinitsin (1914) demonstrated young flukes in the washings from the abdominal cavity of rabbits to which encysted cercariae had been administered and concluded that the flukes must reach the liver through active penetration of the liver capsule; this observation was supported by Shirai (1927). Sinitsin's theory was further supported by Shaw (1932), who injected larval flukes directly into the peritoneal cavities of rabbits, guinea pigs, and lambs and observed that the young flukes penetrated the hepatic

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capsule; juvenile flukes were later recovered from the liver. While the observations of these investigators demonstrated the ability of the excysted metacercariae to gain access to the liver by penetration of the liver capsule, it was not shown that this route is the normal one or that the young flukes on reaching the liver could gain access to the bile ducts and become mature. Since the flukes are sometimes found in such abnormal locations as the lungs and elsewhere, and may even be acquired prenatally, it would seem reasonable to conclude that migration to the liver via the peritoneal cavity was not the usual one. In order to secure additional information on the course of migration of *F. hepatica* in the definitive host, a number of experiments involving the transfer of larval flukes from one definitive host to another were carried out; the results of these experiments are presented in this paper.

MATERIALS AND METHODS

The larval flukes used in the transfer experiments described herein were obtained by administering to white mice and guinea pigs (first definitive hosts) cysts of *F. hepatica* obtained from laboratory infected snails. After a number of days had elapsed, the definitive hosts were killed, the young flukes recovered either from the peritoneal cavity or the liver tissue, and transferred in saline by means of a pipette directly into the peritoneal cavities of guinea pigs, rabbits and sheep (second definitive hosts). Guinea pigs were found to be unsatisfactory for this purpose, as the flukes failed to reach maturity in them. In making the transfers, a surgical incision was made in the test animal in the region of the flank, in the case of abdominal transfers, and between the ribs, in the thoracic transfers, the operative openings being closed by sutures. The operations were carried out either under local or general anesthesia.

EXPERIMENTAL DATA

1. Direct transfer of immature flukes to abdominal cavity of rabbits and sheep

Larval flukes were transferred directly to the abdominal cavities of 20 rabbits and 3 sheep, and the results of these experiments are given in Table 1. The data presented in this table show that young flukes obtained from one definitive host will, when transferred to a second definitive host, reach the liver and become mature in the bile ducts. These data also indicate that the average time for the flukes to reach fertile maturity in rabbits is somewhat less than in sheep, the range being 62 to 99 (average 71) days in rabbits and 79 to 101 (average 86) days in sheep (includes period in first definitive host). Since only three sheep were involved in these experiments it is possible that had a larger number of animals been used the average time required for the flukes to mature might have been slightly less.

The importance of a sufficient flow of bile for the fluke in the bile duct is shown by the data for rabbit 1. These flukes although 88 days old when recovered were still immature, being only 9 and 12 mm long, respectively, when relaxed. They had lodged in the minor, peripheral bile ducts of the lobes of the liver, whereas the flukes which make a normal growth are usually found in the largest ducts.

Usually conspicuous points of entrance of juvenile flukes are discernible on the liver surface. The lesions persist for weeks, and the ability to repair such damage seems to vary considerably with different species. Healing is more rapid and complete in guinea pigs than in sheep and rabbits.

In order to ascertain whether the transfer of immature flukes from one host animal to another affected the rate of maturity, encysted metacercariae were administered *per os* to three rabbits and one sheep. The first rabbit received 11 cysts; eggs appeared in the feces 66 days later and 1 fluke was recovered at necropsy. The second rabbit received 17 cysts; eggs appeared in the feces in 69 days, and six flukes were recovered at necropsy. The third rabbit received 40 cysts; eggs appeared in the feces in 81 days, and six flukes were recovered at necropsy. The sheep (no. 12039) received 130 cysts; eggs appeared in the feces in 75 days, and 21 mature flukes were recovered from the bile ducts when the animal was necropsied 30 days later.

The results of these experiments parallel

those obtained by direct transfer of the immature flukes and show that the time required for reaching maturity is not materially affected by the manipulations necessary during the transfers.

2. Direct transfer of immature flukes to the pleural cavity of rabbits

Since the liver fluke has been reported on a number of occasions from abnormal locations, even under circumstances indicating prenatal infection,[2] the prevailing opinion is that in order for the flukes to reach unusual locations they must be transported by the circulation. In order to secure information on this point, limited experiments were conducted as follows:

Four 30-day-old larval flukes obtained from the liver of a mouse were transferred to the thoracic cavity of a fully grown rabbit. This animal was examined two months later and the thoracic organs appeared normal; examination of the liver, however, revealed a single specimen of *F. hepatica*, 20 mm long by 7 mm wide, in one of the bile ducts. In a second rabbit, about one-fourth grown, two 22-day-old flukes were transferred to the thoracic cavity; this animal was examined a month later and a single fluke 25 mm long by 6.5 mm wide that had just reached maturity was recovered from the liver. A typical entrance point was observed in the liver capsule indicating that the fluke had reached the liver by migration.

A third rabbit, almost fully grown, received by direct transfer into the thoracic cavity four 30-day-old flukes. A week later this animal developed paralysis of the hind quarters and died a week after the symptoms appeared. On examination one lung was found to be hemorrhagic, a portion of

[2] One case of liver fluke infection was observed in the vicinity of Logan, Utah, in a 6-weeks-old calf; the flukes were all mature.

TABLE 1.—RESULTS OF INFECTIONS OF FASCIOLA HEPATICA IN RABBITS AND SHEEP PRODUCED BY THE DIRECT TRANSFER OF IMMATURE FLUKES TO THE ABDOMINAL CAVITY

Animal designation	Source of flukes	Length of flukes	Age of flukes at time of transfer	Flukes transferred	Appearance of eggs in feces[5]	Age of flukes at time of oviproduction[6]	Days in final host—rabbit or sheep	Flukes recovered
		Mm	*Days*	*Number*	*Days*	*Days*	*Days*	*Number*
Rabbit 1	Mouse	—	7	2	0	—	81	2[1]
Rabbit 2	do	—	8	3	66	74	76	1
Rabbit 3	Mice	1.0–1.5	8	2	66	74	73	2
Rabbit 4	Mouse	1.0–1.5	9	2	61	70	66	1
Rabbit 5	do	3.0±	16	3	52	68	55	3
Rabbit 6	do	3.0±	16	11	50	66	63	11
Rabbit 7	do	—	16	5	54	70	66	4
Rabbit 8	do	—	9	2	0	—	78	0
Rabbit 9	Mice	2.0–4.0	20	8	45	65	58	8
Rabbit 10	Mouse	1.0–1.5	8	6	0	—	35	6[1]
Rabbit 11	do	6.5–8.0	28	3	34	62	36	2
Rabbit 12	do	6.5–8.0	28	3	44	72	62	2
Rabbit 13	do	6.5–8.0	28	1	44	72	64	1
Rabbit 14	do	6.5–8.0	28	3	34	62	62	1
Rabbit 15	do	6.5–8.0	27	4	—	—	15	3[1]
Rabbit 16	do	6.5–8.0	30	4	46	76	88	3
Rabbit 17	Mice	—	30	4	—	—	19	2[1]
Rabbit 18	Guinea pig	8.0	29	1	—	—	77	0
Rabbit 19	do	6.0	31	2	—	—	99	2
Rabbit 20	do	6.0	31	3	68	99	68	2
Sheep 12023	Mice	less than 1.0	5	15	74	79	697	—[2]
Sheep 12083	do	1.0–2.0	11	41	90	101	100	10[3]
Sheep 12026	do	2.0–4.0	20	48[4]	59	79	77	17

[1] Immature.
[2] Animal not destroyed, fluke eggs still numerous.
[3] Some flukes immature.
[4] Transfer made through cannula; some young flukes may have been lost.
[5] Days in rabbit or sheep.
[6] Total days in mouse or guinea pig and rabbit or sheep.

the pericardium was thickened and congested, and the thymus was hemorrhagic with adhesions between it and the thoracic wall. No flukes were recovered directly from the organs, but one specimen that showed considerable growth was recovered from the water in which the thoracic organs were manipulated. It is assumed that the paralysis occurring in this case was the result of the fluke infection as no cases of this sort have occurred during several years in the rabbit colony from which this animal was obtained.

In the fourth experiment three 27-day-old flukes were transferred to the thoracic cavity of a mature rabbit. This animal died 22 days after the transfer. On necropsy the parietal pleura in the region of the operative opening was roughened, and there were small hemorrhages in the intercostal muscles. Flecks and strands of fine connective tissue were present on the surface of the lung and areas of scar tissue were observed in the lung tissue which were probably the result of injuries caused by the migrating flukes. The pleural sac was ruptured medially posterior and dorsal to the heart and a portion of the lung had passed through this opening and had become strangulated. The strangulated portion of the lung was consolidated and was showing evidence of necrosis; adhesions and connective tissue deposits were present in this region. No flukes were recovered from this animal.

SUMMARY AND CONCLUSIONS

These experimental data show that, if juvenile flukes reach the peritoneal cavity of rabbits and sheep, they migrate to the liver, penetrate the capsule and parenchyma, enter the bile ducts, and mature. Furthermore, it is shown that entrance through the bile duct is precluded as necessary in the infection of rabbits and sheep; however, it is not eliminated as a possible infection route. In view of the experiments recorded in this paper, particularly those concerning the transfer of flukes to the thoracic cavity, and because of the large size of some of the flukes transferred, the circulatory system as a transfer route also is precluded as being necessary; however, a source of blood seems to be essential for survival; the juvenile flukes are able to secure blood because of their ability to penetrate tissues. Since 78 percent (56 flukes) of the 72 juvenile flukes used in the transfer experiments with rabbits were recovered in necropsies, there is reason to believe that infection via the peritoneal cavity is the principal, if not the sole route, of infection. The limited experiments involving the transfer of juvenile flukes to the thoracic cavity indicate that obscure symptoms of disease, or death, may be traced to liver flukes, even though the flukes themselves may not always be recoverable.

LITERATURE CITED

Bugge, G. *Die Wanderungen der Leberegel in den Organen der Schlachttiere.* Berlin Tierärztl. Wchnschr. **51** (5): 65–68, figs. 1–4. 1935.

Shaw, J. N. *Studies of the liver fluke* (Fasciola hepatica). Journ. Amer. Vet. Med. Assoc. **81**: 76–82. 1932.

Shirai, M. *The biological observation on the cysts of* Fasciola hepatica *and the route of migration of young worms in the final host.* Sci. Rep. Govt. Inst. Infect. Dis., Tokyo, **6**: 511–523. 1927.

Sinitsin, D. F. *New observations on the biology of* Fasciola hepatica. Centralbl. Bakt. I Abt. Orig. **74**: 280–285. 1914.